Amendments to the Specification:

Replace the original Sequence Listing with the substitute Sequence Listing filed herewith.

Please amend the paragraph beginning at page 1, line 13, as follows:

This application is a continuation of U.S. patent application serial No. 09/244,984, filed February 9, 1999, now U.S Patent 6,842,704, which claims the priority benefit of U.S. provisional patent application serial No. 60/073,709, filed February 4, 1998, U.S. patent application serial No. 60/135,499, filed March 30, 1998, and U.S. provisional patent application No. 60/117,476, filed January 27, 1999.

Please amend the paragraph beginning at page 2, line 27, as follows:

According to one aspect of the invention, there is provided a composition comprising a polypeptide in crystalline form, wherein the polypeptide is a TNF-α-converting enzyme polypeptide. In one embodiment, the TNF-α-converting enzyme polypeptide comprises the TNF-α-converting enzyme catalytic domain. In another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the pro and catalytic domains of TNF-α-converting enzyme. In a further embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the amino acid residues 1-477 (SEQ ID NO:8) of TNF-α-converting enzyme. In yet another embodiment, the polynucleotide is substituted such that amino acid residue Ser266 (of SEQ ID NO:8) is changed to Ala and amino acid residue ~~Asn542~~ Asn452 (of SEQ ID NO:8) is changed to Gln, and wherein a second polynucleotide encoding the sequence Gly-Ser-$(His)_6$ (SEQ ID NO:2) is fused to the C-terminus.

Please amend the paragraph beginning at page 7, line 4, as follows:

In a still further aspect of the invention, there is provided a method of identifying a compound that associates with TNF-α-converting enzyme, comprising (A) designing an

associating compound for said polypeptide that forms a bond with the TNF-α-converting enzyme catalytic domain based on x-ray diffraction coordinates of a TNF-α-converting enzyme polypeptide crystal; (B) synthesizing said compound; and (C) determining the associate capability of said compound with said TNF-α-converting enzyme. In one embodiment, the associating compound is an inhibitor, mediator, or other compound that regulates TNF-α-converting enzyme activity. In another embodiment, the associating compound is a competitive inhibitor, un-competitive inhibitor, or non-competitive inhibitor. In still another embodiment, the coordinates are the coordinates of Table 1, or a substantial part thereof. In a further embodiment, the TNF-α-converting enzyme polypeptide crystal comprises the TNF-α-converting enzyme catalytic domain. In still another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the pro and catalytic domains of TNF-α-converting enzyme. In yet another embodiment, the TNF-α-converting enzyme polypeptide is the expression product of a polynucleotide encoding the amino acid residues 1-477 (SEQ ID NO:8) of TNF-α-converting enzyme. In another embodiment, the polynucleotide is substituted such that amino acid residue Ser266 is changed to Ala and amino acid residue Asn542 is changed to Gln, and wherein a second polynucleotide encoding the sequence Gly-Ser-$(His)_6$ (SEQ ID NO:2) is fused to the C-terminus. In a further embodiment, the TNF-α-converting enzyme polypeptide crystal is co-crystallized with a binding partner. In still another embodiment, the binding partner is a hydroxamate-based binding partner or N-{D,L-2-(hydroxyaminocarbonyl)methyl-4-methylpentanoyl}-L-3-amino-2-dimethylbutanoyl-L-alanine,2-(amino)ethyl amide. In yet other embodiments, the TNF-α-converting enzyme polypeptide crystal has a crystal structure diffracting to 2.0 Å, is monoclinic, has a unit cell comprising four crystallographically independent TNF-α-converting enzyme catalytic domain (TCD) molecules, has the TCD molecules are in an asymmetric unit, and/or is of monoclinic space group $P2_1$ and the cell has the constants a=61.38 Å, b=126.27 Å, c=81.27 Å, and β=107.41°. In still another embodiment, ~~the invention~~ the associating compound is designed to associate with the S1' region of TNF-α-converting enzyme. In yet another embodiment, the associating compound is designed to associate with the S1'S3' pocket of TNF-α-converting

enzyme. In still other embodiments of the invention, the associating compound is designed to (i) incorporate a moiety that chelates zinc, (ii) form a hydrogen bond with Leu348 or Gly349 of TNF-α-converting enzyme (SEQ ID NO:7), (iii) introduce a non-polar group which occupies the S1' pocket of TNF-α-converting enzyme, (iv) introduce a group which lies within the channel joining S1' - S3' pockets of TNF-α-converting enzyme and which makes appropriate van der Waal contact with the channel, and/or (v) form a hydrogen bond with Leu348 or Gly349 on the backbone amide groups of TNF-α-converting enzyme (SEQ ID NO:7).

Please amend the paragraph beginning at page 8, line 20, as follows:

Fig. 1: Figure 1 is a ribbon diagram of the TACE catalytic domain (TCD). The chain starts on the lower left back side, runs through the structural elements sI, hAI, hA, sII, hB, hB2, sIII, IV, IVa, sIVb, sV, hC, Met-turn and hD, and ends in the upper left back. The three disulfides are shown as connections, with the sulphurs given as small spheres. The catalytic zinc (central sphere) is liganded by the three imidazoles of ~~His4O5, His4O9~~ His405, His409 and His415 (SEQ ID NO:7), and by the hydroxyl and the carbonyl oxygen atoms of the inhibitor hydroxamic acid group. The inhibitor mimicking interaction of primed-site residues of a peptide substrate is shown in full. Figure 1 was made using SETOR. See Evans, S. "SETOR: Hardware Lighted Three-Dimensional Solid Model Representations of Macromolecules" *J. Mol. Graph.* 11:134-138 (1993).

Please amend the paragraph beginning at page 9, line 15, as follows:

Fig. 4: Figure 4 is a stereo section of the final 2.0 Å electron density around the catalytic zinc (large, central sphere) superimposed with the final TACE model. Visible are the three zinc liganding imidazole rings of ~~His4O5~~ His405 (top), ~~His4O9~~ His409 (left) and His415 (bottom), the "catalytic" Glu406 (all of SEQ ID NO:7), and the hydroxamic acid moiety of the inhibitor. The orientation is similar to Fig. 1. Figure 4 was made using TURBO-FRODO. See Roussel, A. & Cambilleau, C., "Turbo-Frodo in Silicon Graphics Geometry," *Partners Directory*, Silicon Graphics, Mountain View, CA (1989).

Please amend the paragraph beginning at page 11, line 5, as follows:

As used herein, the TACE catalytic domain (TCD) refers to the portion of a TACE polypeptide between residues 215 and 477 (of SEQ ID NO:7) and including the preceding furin cleavage site (residues 211-214), or any part thereof that is capable of cleaving the peptide PLAQAVRSSS (SEQ ID NO:1).

Please amend the paragraph beginning at page 12, line 7, as follows:

In one embodiment of the invention, the cDNA encodes a TNF-α converting enzyme polypeptide comprising the signal peptide, pro and catalytic domains of TACE (TCD), residues 1-477 (SEQ ID NO:8), with Ser266 changed to Ala and Asn452 changed to Gln. These substitutions are useful in preventing N-linked ~~glycosolation~~ glycosylation. Additionally, the sequence Gly-Ser$(His)_6$ (SEQ ID NO:2) may be added to the C-terminus. The addition of the sequence Gly-Ser$(His)_6$ (SEQ ID NO:2) facilitates purification of the polypeptide using metal-chelate affinity resins, such as Ni-NTA resins.

Please amend the paragraph beginning at page 12, line 15, as follows:

Recombinant expression vectors containing the nucleotide sequence encoding TACE, or a portion thereof, may be prepared using well known methods. Suitable host cells for expression of TACE polypeptides include prokaryotic, yeast, and higher eukaryotic cells. Vectors and host cells suitable for use in the present invention are described in WO 96/41624. Further examples of suitable expression systems that can be employed to express recombinant TACE according to the present invention include mammalian or insect host cell culture expression systems, including baculovirus systems in insect cells (See Luckow and Summers, *Bio/Technology* 6:47 (1988))and mammalian cell lines such as COS-7 cells (Gluzman et al., *Cell* 23:175 (1981)). Additional examples are known in the art and include those described in WO 96/41624. In one embodiment of the invention, the TACE polypeptide is expressed in CHO cells. In this

embodiment, the cells secrete a mixture of TACE polypeptide beginning with Val212 and Arg215 (of SEQ ID NO:7).

Please amend the paragraph beginning at page 15, line 27, as follows:

In one embodiment of the invention, a DNA construct comprising TACE residues 1-477 (SEQ ID NO:8), with Ser266 changed to Ala, Asn452 changed to Gln, and the sequence Gly-Ser-$(His)_6$ (SEQ ID NO:2) added to the C-terminus, may be expressed in CHO cells. These cells primarily secrete a processed mixture of TACE, about half beginning with Val212 and about half with Arg215. The mixture is purified as described above. The purified TACE polypeptide, with the added binding partner, is stored in a buffer as described above.

Please amend the paragraph beginning at page 22, line 4, as follows:

The C-terminal chain comprising the last 61 TCD residues (of SEQ ID NO:7) (Fig. 3) first forms three short straight almost perpendicularly arranged segments linked by two "narrow" supertwisted loops, returns via the tight "Met-turn" Tyr433-Val434-Met435-Tyr436 (SEQ ID NO:10) back to the surface where it kinks at Pro437 to form the Pro437-Ile438- Ala439 outer "wall" of the S1' crevice, approaches in a wide loop the C-terminal α-helix hD and runs through it, and ends up on the molecular "back" surface close to the N-terminus, with the last defined residues Arg473-Ser474 fixed via hydrogen bonds to the main molecular body. Via Cys423-Cys453, the first of the two "narrow" loops is disulfide-linked with the N-terminus of helix hD, whose C-terminal end in turn is clamped to the "ear-like" sIV-sV linker peptide through Cys365-Cys469. Spatially adjacent, the third disulfide bridge of TCD, Cys225-Cys333, connects the N-terminal parts of β-strands sI and sIII. In the intact TACE molecule, four residues downstream of Ser474 would reside Cys478, which is already integral part of the compact elongated disintegrin domain (Saudek *et al.*, "Three-dimensional structure of echistatin, the smallest active RGD protein" *Biochem.* 30, 7369-7372 (1991)). Considering Ser474 and this Cys478 as pivot points of their respective domains, the three residue linker would allow

relatively unconstrained docking of the disintegrin domain to the "left" surface side of the catalytic domain.

Please amend the paragraph beginning at page 22, line 22, as follows:

The active-site cleft of TACE (Fig. 2a) is relatively flat on the left hand (non-primed) side, but becomes notched towards the right. The catalytic zinc residing in its center is penta-coordinated by the three imidazole N∈2 atoms of ~~His4O5, His4O9~~ His405, His409 and His415 (of SEQ ID NO:7) (provided by the active-site helix and the following ''descending'' chain comprising the conserved zinc binding consensus motif HEXXHXXGXXH) (SEQ ID NO:3), and by the carbonyl and the hydroxyl oxygen of the hydroxamic acid moiety of the inhibiter (see Figs. 1, 2a and 4). This zinc-imidazole ensemble is based on the distal ∈-methyl-sulphur moiety of the strictly conserved Met435, harbored in the Met-turn characteristic for the metzincin clan (Bode *et al.*, "Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc binding environments (HEXXHXXGXXH (SEQ ID NO:3) and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'" *FEBS Lett.* 331, 134-140 (1993); Stöcker *et al.*, "The metzincins: Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases" *Protein Sci.* 4, 823-840 (1995)). Both carboxylate oxygens of the "catalytic" ~~Glu4O6~~ Glu406 (of SEQ ID NO:7) (which acts as a general base during catalysis (Grams *et al.*, "X-ray structures of human neutrophil collagenase complexed with peptide hydroxamate and peptide thiol inhibitors: Implications for substrate binding and rational drug design" *Eur. J. Biochem.* 228, 830-841 (1995)) squeezed between the zinc-liganding imidazole of ~~His4O5~~ His405 (of SEQ ID NO:7) and the edge strand, are hydrogen bonded to the hydroxyl and the N-H group of the hydroxamic acid (see Fig.4). To the right of the catalytic zinc opens the deep S1' pocket, which, besides the S1' wall-forming segment (bottom, Figs. 1 and 2a), is bordered by the side chains of ~~His4O5~~ His405 and ~~Glu4O6~~ Glu406 (of SEQ ID NO:7) (left), the sIV main chain and the Leu345 side chain (top), and the side chains of ~~Val44O~~ Val440 (back) and Ala439 (right) (of SEQ ID NO:7). To the right of Ala439 opens a second (S3') pocket, which inside the

molecule merges with the S1' pocket, leaving a small bridge made of the opposing side chains of Ala439 and Leu348 (Fig. 2a).

Please amend the paragraph beginning at page 24, line 3, as follows:

The P1' to P3' segment Val77-Arg78-Ser79 (of SEQ ID NO:7) of a bound pro-TNFα probably binds in a similar manner, possibly under better matching with the underlying cleft surface; the preceding P3 to P1 residues Ala74-Gln75-Ala76 (of SEQ ID NO:7) certainly will align antiparallel to the edge strand, with their side chains extending into the (partially charged) S3 pocket and the (negatively charged) shallow S2 depression, and projecting out of the central cleft, respectively. The primed subsites and surrounding molecular surfaces of TACE are dominated by negative charges, while the non-primed subsites are essentially hydrophobic in nature (Fig. 2a). More distant interactions may be involved in the specificity of TACE for processing pro-TNFα. The 12 residue substrate comprising the pro-TNFα cleavage site can also be split by some of the MMPs, although with less specificity and efficacy (Black *et al.*, "Relaxed specificity of matrix metalloproteinases (MMPs) and TIMP intensity of tumor necrosis factor-α (TNF-α) production suggest the major TNF-α converting enzyme is not an MMP" *Biochem. Biophys. Res. Commun.* 225, 400-405 (1996)). Thus, the preferential processing of the (probably trimeric) (Tang *et al.*, "Human pro-tumor necrosis factor is a homotrimer" *Biochem.* 35, 8216-8225 (1996a); Tang *et al.*, "Length of the linking domain of human pro-tumor necrosis factor determines the cleavage processing" *Biochem.* 35, 8226-8233 (1996b)) membrane-bound pro-TNFα in *vivo* might in part be due to correct assembling, i.e. suitable presentation of the pro-TNFα cleavage segment to the TACE active site in a distinct distance from the anchoring membrane. Some experimental evidence (Tang *et al.*, *Biochem.* 35, 8216-8225 (1996a); Tang *et al.*, *Biochem.* 35, 8226-8233 (1996b)) suggests that the cleavage site might not be determined by the cleavage sequence alone, but that also the distance to the base of the compact cone formed by the associated C-terminal segments of three TNFα molecules (Jones *et al.*, "Structure of tumor necrosis factor" *Nature* 338, 225-228 (1989)) plays a role. In a productive TACE-proTNFα complex, the base of this TNFα-trimer cone (into which the disordered N-termini run

up) may be recognized by the "right" side of the TACE catalytic domain (Fig. 2a), with the about 10 residues long spacer favoring the correct placement of the proTNFα Ala76-Val77 (of SEQ ID NO:7) scissile peptide bond in the active site of TACE.

Please amend the paragraph beginning at page 25, line 4, as follows:

The polypeptide topology and in particular the surface presentation of the catalytic zinc prove the catalytic domain of TACE to be a typical metzincin. (Bode *et al.*, "Astacins, serralysins, snake venom and ~~matmrix~~ matrix metalloproteinases exhibit identical zinc binding environments (HEXXHXXGXXH (SEQ ID NO:3) and Met-turn) and topologies and should be grouped into a common family, the 'metzincins'" *FEBS lett.* 331, 134-140 (1993); Stöcker *et al.*, "The metzincins: Topological and sequential relations between the astacins, adamalysins, serralysins, and matrixins (collagenases) define a superfamily of zinc-peptidases" *Protein Sci.* 4, 823-840 (1995)) A superposition with the other metzincins shows, however, that its topology is most similar to that of the catalytic domain of snake venom metalloproteinases such as adamalysin II (Fig. 5). (Gomis-Rüth *et al.*, "First structure of a snake venom metalloproteinase: prototype for matrix metalloproteinases/collagenases" *EMBO J.* 12, 4151-4157 (1993); Zhang *et al.*, "Structural interaction of natural and synthetic inhibitors with the venom metalloproteinase, atrolysin C (form d)" *Proc. Natl. Acad. Sci. USA* 91, 8447-8451 (1994); Kumasaka *et al.*, "Crystal structure of H2-proteinase from the venom of Trimeresurus flavoviridis" *J. Biochem.* 119, 49-57 (1996)) This close homology is reflected by the much better simultaneous superposition of the central sheet and the large helices, but in particularly also by a couple of structural features, which TACE shares exclusively with the adamalysins such as: the long helix hB and the preceding multiple-turn loop arranged on top of the β-sheet; the typically arranged and shaped C-terminal helix hC; and the extended C-terminus placed on the backside surface. About 175 of the 263 TACE and 201 adamalysin α-atoms are topologically equivalent (with an rms deviation of 1.3 Å, 39 of which have identical side chains (Fig. 3). These numbers are close to those obtained from a comparison of members within the different metzincin families. (Stöcker *et al.*, *supra*) In addition, detailed structural features prove the close relationship of

TACE to the adamalysins: a more conserved core structure; the loosely arranged N-terminus; the characteristic Asp416 (directly following the zinc binding consensus motif, Fig. 3) involved in identical intramolecular hydrogen bond interactions; the adjacent disulfide bridge Cys423-Cys453 (of SEQ ID NO:7) linking the first narrow loop to the C-terminal helix hD (which TACE does not share with adamalysin II, but with the H2-proteinase from the snake venom of T. flavoviridis) (Kumasaka *et al.*, *supra*); disulfide bridge Cys365-Cys469 (of SEQ ID NO:7) connecting the sIV-sV linker with the C-terminal helix hD; a similarly shaped active-site cleft, with particularly strong similarities in the SI' pocket and other primed subsites.

Please amend the paragraph beginning at page 26, line 9, as follows:

The catalytic domain of TACE (TCD) also differs from adamalysin II in several respects: with 263 residues, its chain is much longer; most of the additional residues of TACE are clustered giving rise to a more projecting hA-sII turn, to the two surface protuberances of the multiple-turn loop, to the two "ears" of the sIV-sV linker, and to a more bulged-out sV-hC connector (see Figs. 3 and 5); lack of a calcium binding site but presence of a third disulfide bridge Cys225-Cys333 (of SEQ ID NO:7) in TACE, both elements serving, however, for the same function namely to clamp the N- terminal chain to strand sill; the quite deep S3' pocket of TACE which merges with its S1' pocket; an almost inverted charge pattern in and around the primed subsites, with an absolute predominance of positive charges in adamalysin.

Please amend the paragraph beginning at page 28, line 11, as follows:

The compounds that associate with TACE, for example, may be designed to associate with the S1' region or the S1'S3' pocket of TACE. Compounds that associate with TACE also may be designed to (i) incorporate a moiety that chelates zinc. Further exemplary compounds include compounds are designed to form a hydrogen bond with Leu348 or Gly349 (SEQ ID NO:7) of TACE, (ii) introduce a non-polar group which occupies the S1' pocket of TACE, (iii) introduce a group which lies within the channel joining S1' - S3' pockets of TACE and which makes appropriate van der Waal contact with the channel, and (iv) form a hydrogen bond with

Leu348 or Gly349 (SEQ ID NO:7) on the backbone amide groups of TNF-α-converting enzyme, or (v) any combination of the above.

Please amend the paragraph beginning at page 33, line 10; as follows:

A DNA construct comprising the prodomain and the catalytic domain of human TACE (resides 1-477) (SEQ ID NO:8) was fused to the sequence Gly-Ser-$(His)_6$ to facilitate purification of the protein on a Ni-NTA affinity column. Chinese Hamster Ovary (CHO) were cells used for protein expression. The cells secreted a mixture of mature TACE beginning with either Val212 or Arg215. TACE-containing fractions from the Ni-NTA column were incubated in a buffer containing octylglucoside and the binding partner N-[D,L-[2-(hydroxyaminocarnbonyl)methyl]-4-methyl-pentanoyl)-L-3-(tert-butyl)-glycyl-L-alanine. The final purification step was performed on a gel filtration column. Purified TACE was stored in a buffer containing 10 mM Tris/HCL pH 7.5, 100 mM NaCl, 10% glycerol and 1 mM of inhibitor (TACE buffer).

Please amend the paragraph beginning at page 36, line 4, as follows:

Molecules 1 and 2, and 3 and 4 are defined from Asp219 and Met221, respectively, to Ser474 (of SEQ ID NO:7).

Please amend the paragraph beginning at page 76, line 7, as follows:

250 μM peptide substrate (Ac-SPLAQAVRSSSR-NH_2) (SEQ ID NO:9) was ~~incubate~~ incubated with 3.7 U/μL TACE in a buffer containing 10mM TRIS HCl, pH 7.4, 10% glycerol at 25 degrees C. The reaction was quenched with 1% TFA (final concentration) after two hours. The reaction mixture was separated by HPLC on a Hewlett-Packard 1150. The product formation was monitored by absorbance at 220nm.